Form and function of fetal and neonatal pulmonary arterial bifurcations

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Received 18 May 2000; accepted in final form 27 July 2000

Bennett, Stephen H., Marlowe W. Eldridge, Daniel Zaghi, Shaaron E. Zaghi, Jay M. Milstein, and Boyd W. Goetzman. Form and function of fetal and neonatal pulmonary arterial bifurcations. Am J Physiol Heart Circ Physiol 279: H3047–H3057, 2000.—Bifurcation is a basic form of vascular connection. It is composed of a parent vessel of diameter \( d_o \) and two daughter vessels, \( d_1 \) and \( d_2 \), where \( d_o > d_1 \geq d_2 \). Optimal values for the bifurcation area ratio, \( \beta = (d_1^2 + d_2^2)/d_0^2 \), and the junction exponent, \( x \), in \( d_0 = d_1^x + d_2^x \), are postulated to be universal in nature. However, we have hypothesized that the perinatal pulmonary arterial circulation is an exception. Arterial diameters were measured in pulmonary vascular casts of a fetal lamb (140 days gestation/145 days term) and a neonatal lamb (1 day old). The values for \( \beta \) and \( x \) were evaluated in 10,970 fetal and 846 neonatal bifurcations sampled from the proximal and intermediate arterial regions. Mean values and confidence intervals (CI) for the fetus were \( \beta = 0.890 (0.886–0.895 \text{ CI}) \) and \( x = 1.75 (1.74–1.76 \text{ CI}) \); and for the newborn were \( \beta = 0.913 (0.90–0.93 \text{ CI}) \) and \( x = 1.79 (1.75–1.82 \text{ CI}) \). These values are significantly different from Murray’s law (\( \beta > 1, x = 3 \)) or the West-Brown-Enquist law (\( \beta = 1, x = 2 \)). Therefore, perinatal pulmonary bifurcation design appears to be distinctive and exceptional. The decreasing cross-sectional area with branching leads to the hemodynamic consequence of shear stress amplification. This structural organization may be important for facilitating vascular development at low flow rates; however, it may be the origin of unstable reactivity if elevated blood flow and pressure occurs.

pulmonary arterial morphometry; branching complexity; heterogeneity

THE MAMMALIAN FETAL PULMONARY circulation is one of the few examples in nature where arterial vessels have made special structural adaptations in an effort to subserve its function (23, 56). In the fetus, the gas-exchange function of the lung is dormant (19). Compared with an adult, fetal pulmonary arterial vessels possess an increased wall thickness and smaller internal diameters, leading to a very high resistance that serves to shunt blood flow away from the lung to other organs prior to birth (54). The fetal period is also hemodynamically unique because the pulmonary circulation requires only a small amount of blood flow to sustain development (69), but it experiences a large driving pressure that would be considered hypertensive if it persisted after birth (57). At birth, the fluid-filled lung airways expand with air, and concomitantly the pulmonary circulation experiences a 10-fold increase in blood flow as pulmonary arterial vessels dilate and capillaries are recruited to decrease pulmonary vascular resistance (64). Pulmonary arteries continue to remodel after birth with additional increases in arterial diameter contributing to a continuing decline in vascular resistance (54). Although the dramatic increase in pulmonary blood flow at birth, one hemodynamic curiosity of the fetal pulmonary circulation is an unusual sensitivity of pulmonary arterial vessels to endothelial injury and vascular smooth muscle cell remodeling resulting from premature elevations in blood flow (2, 4, 46, 52, 53). Although such flow disturbances are not fatal to the fetus, they can lead to postnatal complications such as persistent pulmonary hypertension and possibly death (48, 49, 60, 61). Although the explanation for this unusual reactivity is not presently understood, it may be related to the complexity of a vascular network organization unique to the fetal state (17, 28, 57).

The manner in which parent and daughter vessel diameters are connected at a bifurcation would be an elementary way in which network organization could contribute to the unique hemodynamic properties of the fetal pulmonary circulation (55, 76, 77). In a bifurcation, it has been long recognized that flow travels through the diameter of the larger parent vessel (\( d_o \)) connected to two smaller diameter daughter vessels (\( d_1 \) and \( d_2 \)) with flow adhering to a local power-law scaling relationship (65)

\[
d_0^x = d_1^x + d_2^x
\]

where \( d_o > d_1 \geq d_2 \) and \( x \) is the junction exponent. Bifurcation form can be summarized by three factors, the area ratio, \( \beta \) (11), the asymmetry ratio, \( \gamma \) (40, 55),

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and the junction exponent, $x$ (65). Upon defining the asymmetry ratio between daughter vessels as $\gamma = d_2/d_1 \leq 1$, the area ratio, $a$, becomes (55)

$$\beta = \frac{d_1^2 + d_2^2}{d_0^2} = \frac{1 + \gamma^2}{(1 + \gamma)^{2x}} \quad (2)$$

According to Fig. 1, the value of $x$ is pivotal in controlling divergent dependencies on the relationship between the area ratio and branching asymmetry, that is, for $x > 2$, $\beta$ increases with increasing $\gamma$; when $x = 2$, $\beta$ is independent of $\gamma$; and when $x < 2$, $\beta$ decreases with increasing $\gamma$. Consequently, bifurcation form has the potential to influence its physiological and pathological function in a variety of ways, either by changing the distribution of flow, adjusting the pressure drop, or by regulating hemodynamic forces, such as wall tension and shear stress, via changes in vessel diameter and vessel wall morphology. However, despite this potential for variation, only three basic bifurcation designs for $x$ have been postulated to be universal for transport networks in nature, whereby a given design reflects an alternative principle of economy formulated mathematically as a law. One design, according to Murray’s law (50), postulates a universal condition of $x = 3$ and $\beta > 1$, based on principles of constant wall shear stress and minimum power (43). Another law, developed by Kurz and Sandau (41) postulates that $x = 2.7$ and $\beta > 1$ based on the principle of constant wall tension. Alternatively, the West-Brown-Enquist law postulates that $x = 2$ and $\beta = 1$, a condition leading to constant blood flow velocity subject to an allometric relationship of blood flow delivery and basal metabolic rate (73). In contrast, we argue that the unusual properties of the fetal pulmonary circulation can be accounted for by a radical conjecture that many bifurcations are organized according to $x < 2$ and $\beta < 1$, a design for vascular systems that can be potentially unstable at lower thresholds of elevated blood flow. Although the morphometric characteristics of fetal and neonatal vessels have been well documented (54), the form of their bifurcation design is not known. Therefore, the purpose of this report is to measure bifurcation diameters in the fetal and neonatal pulmonary circulation and to assess alternative design hypotheses.

**THEORY OF BIFURCATION DESIGN: ECONOMY, INTEGRATION, AND ADAPTATION**

To understand the grounds for an alternative design hypothesis, it is instructive to recapitulate the premises upon which the universal bifurcation design theories are based and reevaluate their applicability to the form and function of the fetal pulmonary circulation. In general, universal theories are built upon the premise that all vessels are optimally designed to carry flow in some sense. In theoretical terms, optimality has been formulated using mathematical principles of economy. Bifurcation design is hypothesized to accommodate blood flow in the context of a cost function, which optimizes a given measure of maximum efficiency at a minimum of energetic cost (50, 66). However, physiological design principles should also be expected to include factors of integration and adaptation (71) where mathematical principles of optimality are not necessarily the only conditions being satisfied. For example, while vessel elements making up bifurcation demonstrate simple behavior, their integration into bifurcations and networks may lead to complex behavior, not predicted by the system elements themselves (17, 71). Therefore, it is instructive to reexamine how bifurcation complexity can modulate hemodynamic forces, especially since we now know that these forces have the capacity to modulate vessel diameter over different time scales and spatial scales (13, 15, 23). Also, universal theories, which postulate specific values for bifurcation design, predispose one to assume that such values are fixed before birth either genetically (70) or phenotypically in combination with a given hemodynamic signal such as shear stress (43), wall tension (41), or blood flow velocity (73). However, a design based on optimum economy for blood flow does not anticipate situations, such as the fetal pulmonary circulation, where vessel form and function may be quite different from one intended for transport vessels in general (54, 56). Also, although universal theories of...
economy predict more than one bifurcation design to be prevalent in nature, they do not presently offer explanations for bifurcation design variation (16, 42, 55, 62, 75). Here, evidence of an underlying variation may represent the functional capacity of bifurcations to adapt their design (71) to match form and function in response to a much broader spectrum of hemodynamic signals than the simple ones constrained by constant shear stress, wall tension, or blood flow velocity.

Economy. Bifurcation designs leading to postulated universal values for $x$ have as their foundation an underlying premise that vessel form and function in blood flow transport networks are organized on principles of economy (50, 66, 71). Investigation into the relationship between a vessel’s diameter and the magnitude of blood flow that it carries has had a long history of interest. James Keill, in 1708, postulated a bifurcating branching structure to estimate the blood flow velocity in capillaries (74). Later, according to the review of Kurz et al. Roux, in his thesis of 1878 (40), was perhaps the first to draw attention to the fact that physical forces influence the form of bifurcations, imposing constraints on function and shape that cannot be circumvented by genetic or regulatory means. He calculated stem-branch diameter relationships for $\gamma$ and branching angle, and addressed $x$ as a fundamental parameter characterizing bifurcation form and function. By 1901, Thoma’s studies (65) in blood flow led him to conclude that the physiological range for $x$ was from 2–4. Hess (30), in 1913, hypothesized that in all parts of the arterial tree there should be a vessel size where the total energy cost of transporting blood via vessel resistance is economized by a minimum in its relationship to its cross-sectional area. From such a minimum, he predicted that the size of vessels must be reduced with branching by a factor of $\frac{3}{4}$. However, it was Murray (50) who first postulated that one of the fundamental principles of economical physiological organization was based on the quantitative principle of minimum work. Murray proposed that flow $Q$ was proportional to diameter according to a local power law, $Q = kD^x$, where $k$ is a constant and $x$ is an exponent, almost always positive. Murray demonstrated that there are two basic power “costs” associated with moving blood through arteries. One cost component is the pumping power of blood, that is proportional to $(Q^2/D^4)$ times length, while the other is a power cost proportional to a metabolic cost times volume. Murray’s insight lies in recognizing that the total power is minimized when an optimal tradeoff occurs between viscous power dissipation and metabolic power loss. Murray demonstrated mathematically that the optimum condition occurs when $x = 3$. This optimum is distinctive because it holds for all flow rates and scales with vascular systems of all sizes (58). Thus the elegance of this result and the effect of the organizational principle of minimum work on physiology cannot be underestimated. D’Arcy Wentworth Thompson (66) emphasized quite strongly in his book *On Growth and Form* that the principle of minimum work is the mechanism “that is best possible under all circumstances.” He also states that to “believe it to be so is part of our common faith in the perfection of Nature’s handiwork” and that this mechanism should serve as a “postulate, or methodus inveniendi, and it does not lead (the physiologist) astray” (66).

However, vascular systems do demonstrate bifurcation design deviations from the minimum work principle (3, 16, 42, 55, 62, 75). Furthermore, several other investigators have generalized Murray’s law and have deduced alternative minimal cost functions that could potentially describe the diversity of bifurcation branching angles, their area ratios, their asymmetry, and their values of $x$ in nature. Uylings (67) generalized the diameter scaling relationship involving $x$ to reflect alternative conditions of flow and concluded that the optimal value of $x$ ranged from $x = 2.33$ for turbulent conditions to $x = 3$ for laminar flow. Sherman (58) provided a generalization of Murray’s optimization between vessel volume and power that applies to the optimization of any vessel network and does not depend upon minimizing a metabolic cost function. Zamir (76) considered other cost functions that could be physiologically minimized under conditions of flow $Q = kd^x$, such as minimum lumen surface area, minimum drag force arising from shear stress, minimum lumen volume, and minimum power loss. Roy and Woldenberg (55) extended Zamir’s results to a generalized flow condition of $Q = kd^x$. They found that the models which minimize a geometric parameter, such as surface area ($x = 2$), or volume ($x = 3$), are sensitive to variations in $x$ in a different way from those that minimize flow-related parameters such as power loss due to viscous friction and shear stress. Moreover, Woldenberg and Horsfield (75) determined that certain ranges of $x$ are associated with different optimality criteria: the minimization of the surface area lumen is best represented by $0.7 < x < 2.1$; minimum volume occurs in the range $2.1 < x < 2.8$; minimum power optimization is best represented by $2.8 < x < 3.5$; and the range $3.5 < x < 4$ can be described by minimum power or minimum surface area. Griffith and Edwards (27) subsequently determined that the values of $x$ predicted by Zamir (76, 77) and Woldenberg and Horsfield (75) are physiologically adjusted to the simultaneous minimization of different optimal conditions: $x = 4$ minimizes both volume and drag; $x = 3$ either volume and power or surface and drag; $x = 2.5$ minimizes surface area and power loss; and $x = 2$ minimizes both drag and power loss. Thus, by adjusting the value of the junction exponent $x$ in Eq. 1, there are several alternative ways to optimize a bifurcation to match a desired geometric, flow, or hemodynamic signal condition.

The economy of vessel form and function extends to the organization of bifurcations into more complex networks, but the optimal designs that are predicted to emerge as universal tend to center on regulatory mechanisms associated with simple hemodynamic signals, such as shear stress, wall tension, or blood flow velocity. In vascular systems, blood flow through arteries imparts physical forces to the vascular wall that can be resolved into two principal components (15). One force...
is pressure acting normal to the vessel wall, which imposes a circumferential stress. The second force is shear stress, a frictional force acting tangentially at the interface between blood flow and the vessel (13, 14). LaBarbera (43) argued that shear stress is a fundamental regulatory signal for which the Murray’s law condition of \( x = 3 \) represents a local set point. Consequently, elementary networks with this form of control would automatically produce a system globally minimized for minimum power dissipation during morphogenesis, growth, and development (43, 58). The \( x = 3 \) condition appears to be selected for across divergent phyla (42), suggesting that this design is likely convergent and universal, representing a common end point in evolution (68). However, in systemic mammalian arterioles, the value of \( x \approx 2.7 \) is observed (62), consistent with a self-organizing adjustment to conditions of constant wall tension in vascular networks, as demonstrated by Kurz and Sandau (41).

Alternatively, West et al. (73) formulated a different branching law proposed to be universal. This law predicts that the diameters of arterial vessels posses an area-preserving design of \( \beta = 1 \) and \( x = 2 \), in accord with a 3/4 allometric scaling law between basal metabolic rate and body weight, a condition approximated by the human pulmonary circulation (34). Lighthill (44) argued that by controlling the area ratios of bifurcations in the systemic circulation (11) to a value of \( \beta = 1 \) and \( x = 2 \), the design prevents unstable flow separation in larger vessels carrying flow at large Reynolds numbers. Also, this design predicts that blood flow velocity in arteries is constant down to exchange vessels, a condition that is also considered optimum under transport conditions (17). Thus, although alternative universal design theories predict common values for \( x \) in nature, it is presently unclear as to why one single design is not preferred over another.

Integration and adaptation. Bifurcations in nature demonstrate a wide variation of values of \( x \) within and between different vascular systems (16, 42, 62, 75). This variation is not necessarily explained by a single cost function, and universal theories offer little explanation for such phenomena. This gap in explanation may be related to constraints imposed by premises made during the formulation of such theories. Although bifurcation design is based mainly on premises related to mathematical principles of economy (50, 66), other design factors such as integrative complex hemodynamic behavior (17, 71) and the functional capacity of real vessels to adapt to different hemodynamic signals (23, 71) may take part in design variation.

One premise of universal design theories deals with branching complexity and its integrating effect on the relationship between bifurcation form and function (17, 71). Classic universal theory idealizes vessel branching as symmetric (30, 51, 66), where the properties of the vessel element possess an optimal design as the sole determinant of hemodynamic function; however, in vascular systems such simplifications are not necessarily true (14, 55, 62). In essence, the otherwise simple behavior of shear stress in idealized vessels demonstrates complex behavior when vessels are connected. It should be appreciated from Table 1 (32, 45) that while certain values of \( x \) do match certain hemodynamic conditions between parent and daughter vessels as prescribed by optimization, given values of \( x \) and \( \gamma \) can also influence the scaling of other flow conditions between parent and daughter vessels. For flow itself, \( x \) and \( \gamma \) influence only the degree of flow partitioning between daughter vessels. However, for other flow-related properties, such as Reynolds number, flow velocity, and shear stress, the value of \( x \) is instrumental in determining the degree of amplification or deamplification between parent and daughter vessels. In symmetric bifurcations, where \( \gamma = 1 \), values of \( x < 3 \) have the potential of amplifying shear stress in the daughter vessels relative to the shear stress of the parent vessel (11, 59). In asymmetric bifurcations, where \( \gamma < 1 \), amplification is further accentuated along the minor daughter pathway. Furthermore, mathe-
mational network models demonstrate that when asymmetry and diameter randomization are introduced into bifurcations of an otherwise symmetric network, where $x \approx 3$, blood flow behavior in the network occurs in a way that cannot be predicted from the behavior of the vessel elements alone (17, 59). Along certain pathways, fluctuations in shear stress are amplified, whereas along other pathways shear stress is attenuated (17, 59). In addition, as $x$ decreases successively to values smaller than 3, the average value of shear stress is spatially amplified to larger magnitudes within the smaller diameter vessels of peripheral branches (59). Such complexity phenomena in models suggest that the integration of branching complexity in vascular systems is accompanied by a broad spectrum of heterogeneous shear stress signals to which the endothelium and smooth muscle must respond. Consequently, as shear stress is a hemodynamic signal capable of modifying vessel diameter and vessel wall function over various time scales (13), the resulting complexity may provide a basis for explaining the tremendous spatial heterogeneity observed in form and function in arterial vessels (12, 24, 25, 35, 36).

Another premise of universal theories is that bifurcation design is fixed before birth, either genetically or in response to a predominant hemodynamic signal acting as a selection factor of bifurcation design (41, 42, 70–73). Consequently, for $x = 3$ the signal is shear stress (43), for $x = 2.7$ it is constant wall tension (41), and for $x = 2$ it is constant blood flow velocity under conditions of a basal metabolic rate (73). In any case, the design selection is considered significant in influencing species survival (41, 43, 73). However, in nature, physiological systems are also selected for by their functional capacity to respond and adapt to a variety of stimuli acting as stressors over different time scales (23, 71). In this regard, universal theories have not anticipated the possibility that the form and function of vessels during development may satisfy a different physiological design criterion than that of the adult (54). Consequently, vessel diameter and bifurcation design adaptation at birth may represent additional selection factors for mammalian species survival (19, 56, 57, 60) where shear stress and wall tension are ever-present forces regulating local physiological and pathological responses over both short-term and long-term time scales (14). This regulation also has a spatial component over dimensional scales ranging from the network level down to the individual cell nucleus (15). Under these circumstances, the biological variation of designs seen in nature can be potentially interpreted as an adaptive history of branching complexity which arises from a broad spectrum of hemodynamic signal information (16, 42, 62).

Fetal/neonatal bifurcation design. A basic argument for a bifurcation design radically different from those predicted by universal design theories is based upon physiological necessity: the pulmonary circulation before birth is not a functional transport organ expected to optimize its design according to an idealized cost function (41, 43, 73). Instead, the gas exchange organ is dormant, subsisting via a small and sufficient blood flow for its development (69). Also, it is important to emphasize that a very narrow range of flows delivered to the fetal pulmonary circulation is necessary to ensure a successful adaptation at birth (2, 47, 52), because beyond an undetermined low-flow threshold pulmonary arterial vessels change their structure and function adversely (4, 7–10, 46–48). Hence, if such a design is radically different from an adult, fetal bifurcations must possess the functional capacity to adapt to another design after birth to accommodate to the dramatically different hemodynamic conditions. Therefore, just as the function of the fetal pulmonary circulation prior to birth appears contrary to that which is intended for general optimal vascular transport networks in nature (41, 43, 73), it is now reasonable to justify that that the form of fetal pulmonary bifurcations are likewise exceptional: instead of $\beta \geq 1$ and $2 \leq x \leq 3$, fetal bifurcations are hypothesized to satisfy an alternative condition of $\beta < 1$ and $x < 2$.

A fetal bifurcation design of $\beta < 1$ and $x < 2$ leads to several hemodynamic consequences, most of which are economical only for the fetal state. One consequence of lower values of $x$ is that the magnitude of the principal resistance within the pulmonary circulation is enhanced (16, 17). This design is spatially expanded to other vessels, thereby increasing the loci of viscous hydraulic energy dissipation. A spatially expansive increase in resistance represents a plausible design condition for a high-resistance shunt, intended to divert blood flow to other organs (56). In addition, with $\beta < 1$, a continuously branching cascade of decreasing cross-sectional area leads to shear stress amplification (6). As shear stress demonstrates a spectrum of actions on the endothelium and smooth muscle over different magnitudes and time thresholds (14), it is plausible that this design would be instrumental in influencing pulmonary vascular development at very low flow rates (69). Not only would this design place fetal distal arterial shear rates closer to their postbirth values, it would thereby reduce the likelihood of endothelial damage near the gas exchange region when the surge in blood flow occurs at birth (29). Also, at birth, when blood flow increases, such a network design would be effective in a more rapid transduction of shear stress and its effect on increasing arterial diameter via flow-dependent endothelial mechanisms (1, 19, 20). However, it must be emphasized that this design confers an organizational form of structural reactivity (23) that is potentially unstable, whereby excessive blood flow to the fetal pulmonary circulation above a low-flow threshold could induce peripheral arteriolar endothelial dysfunction secondary to a shear stress injury (7–10, 21, 48, 52, 53).

METHODS

Cast preparation. The diameters of the extant fetal and neonatal pulmonary circulation were evaluated via lung casts. Under an approved animal use protocol at the University of California Davis, a pregnant ewe with a fetus of 140 days gestation, along with a 1-day-old newborn lamb, were
euthanized by an overdose of pentothal. A thoracotomy was performed, the trachea was clamped, and the lungs were removed en bloc. A 4.5-French cannula was inserted into the pulmonary artery. Fetal airways remained filled with amniotic fluid. The newborn airways were expanded using saline under a hydrostatic pressure gradient of 20 cmH$_2$O via a tracheal cannula. The pulmonary arterial circulation was washed free of blood by saline perfusion. Methyl methacrylate plastic (Coe Tray Plastic; GC America, Chicago, IL) was then injected into the pulmonary arterial system slowly via a syringe over a 2-min period. The setting time of the plastic is 15 min, with dramatic increases in viscosity preventing plastic infusion by 7 min. The plastic was allowed to polymerize overnight, whereupon the lung tissue was macerated in a 20% KOH bath for 3–5 days. The remaining tissue was washed away gently with distilled water, and the cast was allowed to dry.

**Diameter measurement and calculations.** Bifurcation diameters were measured using a video micrometer. The video micrometer consisted of a Macintosh IIci computer equipped with a Data Translation model DT-2255 image frame-grabber board connected to a Zeiss stereomicroscope fitted with a Panasonic model WW1500X video camera. Images were acquired and analyzed via a program Object Image developed by Norbert Vischer (http://simon.bio.uva.nl). Object Image is an extended version of the program, NIH Image (http://rsb.info.nih.gov/nih-image), allowing diameter measurements and derived calculations to be recorded into a database. To measure diameters, the three-dimensional branching aspect of each cast was broken into pieces to facilitate placement of bifurcations onto a plane. Assuming vessels were circular, vessel diameter was calculated as the average of two diameter measurements per vessel segment. Vessel diameters were measured at bifurcations, consisting of a parent vessel, $D_0$, and two daughter vessels, $D_1$ and $D_2$, where $D_0 > D_1 \approx D_2$. From bifurcation diameters, the diameter junction exponent, $x$, of the equation $(d_1/d_0)^x + (d_2/d_0)^x = 1$ was solved by iteration (34), while the area ratio, $\beta$, was calculated according to Eq. 2.

**Statistical analysis.** We assumed that the bifurcations sampled from the lung casts were taken from a dichotomously branching network with self-similar (17, 39, 70–72) and statistically self-similar branching properties (63). Self-similarity and statistical self-similarity are postulates that establish a null hypothesis of universal network design for all bifurcations (41, 43, 73) that can be tested via analysis of variance methods using bifurcation sampling techniques (16, 17). Our null hypothesis is that if the design properties of lung branching are postulated to adhere to fixed universal laws following the principle of self-similarity (17, 71–73), then the averages of $\beta$ and $x$ should be identical, in a statistical sense, at all levels of branching. The additional postulate of statistical self-similarity (63) assumes that the variences of $\beta$ and $x$ are also equal and independent of branching level, even if alternative methods are used to categorize vessel diameters into topologically related groups (17, 39), such as by ranks (17), generations, or orders (33).

We categorized parent diameters of bifurcations into independent “bifurcation levels” (3), so that vascular systems with different distributions of parent diameters could ultimately be related to a canonical self-similar branching model (3, 17) that satisfies the requisite assumptions for an ANOVA (78). The data set consisted of parent diameter, $d_0$, area ratio, $\beta$, and junction exponent, $x$, which were derived from the parent vessel’s corresponding daughter vessels. The resulting data set was then sorted from the largest parent diameter down to the smallest diameter, by levels $k = 0, 1, \ldots n$ categorized according to $R_{d}^{-k} \geq (d_2/D_0) > R_{d}^{-k+1}$. Here, $d_0$ is a parent diameter of a bifurcation in the sorted list, $D_0$ is the diameter of the main pulmonary artery, and $R_d$ is an average diameter ratio ($R_d = d_0/d_1$) computed from the pool of bifurcations and was set to 1.315. The ranking procedure can be thought of as yielding an equivalent asymmetric branching network, whose branching properties are summarized by a diameter ratio, $R_d$, of the major daughter pathway (parent diameter to largest daughter diameter) (17, 32). The ranking procedure results in a nonoverlapping range of parent diameters for each bifurcation level, where each level is assumed to be statistically independent from another, and where the transformed values [atan $\beta$ and atan $x$ (arctangents, expressed in radians)] possess a statistically self-similar Gaussian distribution with bifurcation level (63). The

**Fig. 2.** Numbers of bifurcations (left) along with their corresponding average parent diameter (right) expressed as a function of bifurcation level ($k = 0, 1, 2, \ldots n$) derived from when bifurcations are categorized according to the parent diameter scaling relationship $R_{d}^{-k} \geq (d_2/D_0) > R_{d}^{-k+1}$. $R_d$ is an average diameter ratio ($R_d = d_0/d_1$) calculated from all bifurcations set to $R_d = 1.315$. Level $k = 0$ is main pulmonary arterial bifurcation. Left: $N_k$, bifurcations/level. Right: corresponding average diameter vs. bifurcation level. Newborn vessels are $–1.8$ times larger than fetal vessels at all bifurcation levels studied. ANOVA was performed on levels 0–13, but additional numbers of bifurcations were sampled in the fetus to levels 14–19.
two-factor ANOVA was evaluated on arctangent transformed values of $\beta$ and $x$ using Statview 4.5. Results are reported as mean values along with their associated 95% confidence intervals (CI) for transformed (atan $\beta$ and atan $x$) and their inverse-transformed variables [i.e., $\beta = \tan(\text{atan} \; \beta)$]. Non-overlapping confidence intervals for $\beta$ and $x$ within bifurcation levels were considered to represent statistically significant difference at a $P < 0.05$ level of significance.

RESULTS

Diameter measurements were made in 10,970 fetal and 846 neonatal pulmonary arterial bifurcations. Figure 2 summarizes how the numbers of bifurcations, along with their parent diameters, vary with bifurcation level relative to the main pulmonary artery. The range of diameters include the proximal and distal zones of the pulmonary arterial circulation. In the case of the fetal pulmonary cast, some parent vessels proximal to capillaries were sampled. The Fig. 2, left, indicates that the numbers of bifurcations within a level increase toward smaller diameter vessels as a result of the ranking procedure. In general, from the main pulmonary artery (level 0), down to the smallest diameter compared (level 13), newborn vessels were ~1.8 times the diameter of fetal vessels. Figure 3 shows the histograms for the pooled fetal and neonatal estimates for transformed $\beta$ (left) and transformed $x$ (right) compared with their Gaussian fit. Figure 4 illustrates the relationship among $\beta$, $\gamma$, and $x$ in the fetal and neonatal state summarized as a bivariate distribution (55). The averages for $\gamma$ were nonnormally distributed, with the fetal mean values $\bar{\gamma} = 0.62 \pm 0.23$ SD (arithmetic), 0.59 (geometric), and 0.56 (harmonic), while the newborn mean values were $\bar{\gamma} = 0.56 \pm 0.21$ SD (arithmetic), 0.51 (geometric), and 0.46 (harmonic). Figure 4 demonstrates that fetal and neonatal vessel daughter diameters in the lamb possess a locus of design concentrated on $x < 2$ and $\beta < 1$, where many bifurcations with the same design are found in the adult human pulmonary arterial tree (55).

Figure 5 illustrates the distribution of $\beta$ and $x$ as a function of bifurcation level. For bifurcations levels 0–13, ANOVA demonstrated a slight but significant difference between the fetal and neonatal group alone: for $\beta$ ($F = 6.51, P = 0.011$): fetus mean $\beta = 0.890$ (0.87–0.90 CI); newborn mean $\beta = 0.913$ (0.90–0.93 CI), or for $x$ ($F = 1.812, P = 0.173$): fetus mean $x = 1.75$ (1.74–1.76 CI); newborn mean $x = 1.79$ (1.75–1.82 CI). ANOVA showed a significant difference between levels 0–13 for both lungs [for $\beta$ ($F = 174.98, P < 0.0001$) and $x$ ($F = 18.17, P < 0.0001$)], indicating that $\beta$ and $x$ are different between bifurcation levels. In addition, ANOVA demonstrated a significant interaction between group (fetus vs. newborn) and bifurcation level 0–13 for both $\beta$ ($F = 2.98, P < 0.0002$), indicating that the fetus and newborn manifest differences in design in branching toward smaller peripheral diameter vessels. In both the fetal and neonatal lungs studied, the larger diameter parent vessels (levels 1–7) were consistent with the West-Brown-Enquist law of $\beta = 1$ and $x = 2$. However, beyond level 7, bifurcations followed a fetal pattern of $\beta < 1$ and $x < 2$, both decreasing with increasing branching level away from the main pulmonary artery. Figure 5 reports additional numbers of smaller bifurcations in the fetus beyond level 13, down to a bifurcation level of 19, where from Fig. 2, the parent diame-

Fig. 3. Population histograms of pooled estimates ($N = 11,816$ bifurcations) for arctangent (atan) transformed values of $\beta$ (left: $\beta = 0.891$ [0.672–1.171 confidence interval (CI)]), and $x$ (right: $x = 0.891$ [1.22–2.72 CI]): estimates for $x$ are closer to Gaussian distribution than estimates for $\beta$. Gray areas mark regions of “fetal” bifurcation design ($\beta < 1$ and $x < 2$).

Fig. 4. Area ratio ($\beta$) and asymmetry ratio ($\gamma$) of fetus (left) and newborn (right) expressed as a bivariate frequency distribution. Gray scales represent the numbers of bifurcations within a particular range of $\gamma$, $\beta$, and $x$. Average $\bar{\gamma}$: fetus = $0.62 \pm 0.23$ SD; newborn = $0.56 \pm 0.21$ SD. The equation of the lines is given by Eq. 2 in text.
Fig. 5. Distribution of $\beta$ and $x$ as a function of bifurcation level. Left: transformed scale of $\beta$. Right: transformed scale of $x$ used in ANOVA. Gray area demarcates fetal design values for $\beta$ and $x$. Bifurcation levels 11–13 are common to both fetus and newborn where ($\beta < 1, x < 2$). However, newborn exhibits more levels of fetal design, but the fetus studied showed more dramatic change in design with increasing levels. Overall, proximal levels show bifurcation design behavior consistent with West-Brown-Enquist law ($\beta = 1, x = 2$), but both systems manifest “fetal-like” bifurcation designs ($\beta < 1, x < 2$) in distal bifurcation levels that are heterogeneous with branching.

**DISCUSSION**

The null hypothesis anticipated that fetal vessel connectivity was optimized, either for constant shear stress, constant wall tension, or constant flow velocity, as postulated by universal laws predicted by biological network theory. Such a hypothesis stipulates that bifurcation design criteria satisfy $\beta \geq 1$ and $2 \leq x \leq 3$ for all levels of branching. Under these circumstances, bifurcation design in the fetal, neonatal, and adult state would be identical and homogeneous at all levels of branching. Consequently, differences in the fetal and neonatal state could be attributed solely to vessel geometry alone, concordant with a simple diameter increase due to a vasodilation mechanism after birth (19). However, the results of this study support the alternative hypothesis that the form of bifurcation design in small arterial vessels of the fetal and neonatal pulmonary circulation is distinctive, with $\beta < 1$ and $x < 2$. Furthermore, this bifurcation design is not homogeneous with a uniform distribution with branching but instead shows a serial spatial heterogeneity. These results implicate an alternative form of network organization at the bifurcation and network level different from that previously supposed by universal network theories that assume a fixed homogeneous adult arterial bifurcation design of $\beta = 1$ and $2.0 < x < 2.8$ for the several adult species that have been studied (16, 34). The configuration of fetal bifurcations confirms a hypothesis made by Hopkins (31) that the perinatal pulmonary circulation dissipates hydraulic energy in a manner that is quite different than other stages of pulmonary growth and development and different organ systems (31, 37). However, whereas Hopkins addressed a particular mechanism related to pulsatile power dissipation in the viscoelastic vessel wall, this study identifies an underlying organizational form of structural reactivity (23) that was not previously anticipated by any previous theory of bifurcation design (41, 43, 73). This form of reactivity is embodied by the way vessels are interconnected and would suggest that the fetal and neonatal configuration must remodel substantially during postnatal development by changes in bifurcation complexity to expand the range of flows permissible without injury (34, 55). While the overall average values for $\gamma, \beta,$ and $x$ are much higher in the adult ($\gamma \approx 0.8, \beta \approx 1.0, x \approx 2.3$), fetal-like bifurcation designs appear with relatively high frequency (55). In this regard, our Fig. 4 should be compared with figure 2 of Roy and Woldenberg (55) where several bifurcations in the adult show a similar pattern of design as our fetal and neonatal lamb bifurcations, with $\beta < 1, x < 2,$ and $\gamma \approx 0.3–0.4$. The appearance of excessive numbers of fetal-like bifurcation designs in the adult may therefore be suggestive of a remodeling process due to a prenatal injury (26) or may indicate an incomplete adaptive remodeling process during growth and development. In either case, remnants of fetal bifurcation design in the pulmonary arterial circulation may play a role in predisposing some individuals to endothelial injury at elevated blood flow (18).

The acceptance of these results and implications must be viewed carefully in light of the present study limitations related to casting methods. One important limitation with casting methods (62) is that arterial diameters are not observed directly (16) and rely on an apparent diameter filled by the hydraulic conditions of a high-viscosity polymer (16). Although the resulting diameters are presumed to mimic the extant vessel morphology, the polymerization process is known to be subject to diameter distortion (62). Casting results in a vessel distention error, reported to scale allometrically with vessel radius, where the absolute error in diameter measurement propagates to the smallest vessels. Suwa and colleagues (62) estimated that for vessels from casts in the range 3,000 $\mu$m down to 10 $\mu$m, the scaling follows the power-law $r = 1.369 r_0^{0.9605}$ where $r$ is the radius of resin fixed vessels of the arterial cast and $r_0$ is the corresponding radius of Formalin-fixed vessels. Consequently, for the smallest 30-$\mu$m parent diameter fetal vessels observed in this study, this relationship predicts that they may be over-distended by a factor estimated to be up to 25%. However, it must be
emphasized that we looked only at diameters within a bifurcation using measurements that normalized their values. Thus the error rates due to distension should be distributed equally among the vessels within a bifurcation. A greater possible source of error contributing to the uncertainty in $\beta$ and $x$ arises from the violation of our assumption of cylindrical vessel shape from our method of measuring diameters (38). If such diameter errors were present and random, then they were compensated for in using a large number of bifurcations at different levels of branching. Despite these limitations, casting methods, utilized widely in the adult pulmonary circulation, lead to consistent findings for bifurcation design for several species and correlate well with direct visualization techniques (16). However, in using our bifurcation sampling technique, the connective branching topology of the entire pulmonary arterial tree was lost, so we cannot compare the mean and variance properties of our categorization with the entire pulmonary circulation. Consequently, the results for bifurcation design for several species and correlating with direct visualization techniques (16). However, in using our bifurcation sampling technique, the connective branching topology of the entire pulmonary arterial tree was lost, so we cannot compare the mean and variance properties of our categorization with the entire pulmonary circulation. Consequently, the results for bifurcation design for several species and correlating with direct visualization techniques (16).

In view of these limitations, the results of this study emphasize an elementary concept of branching complexity: individual vessels, with an assumed known form and function, may manifest quite different emergent scaling behavior when connected (17). This study identifies an example in nature where bifurcations are not necessarily endowed with a design based on theoretical principles of economy (43, 66, 73). We must emphasize that the fetal and neonatal pulmonary circulation are not necessarily the only examples where bifurcation values for $\beta < 1$ form elements of branching design in arterial trees (3, 11, 55, 75). However, in the case of the fetal and newborn pulmonary arterial system, this connective behavior provides a new perspective to explain hemodynamic phenomena underlying the process of adaptation or maladaptation during pulmonary vascular growth and development. In effect, branching design may originate from a state far removed from a theoretical one optimized for minimum energy dissipation, as proposed by Thompson (66), and still be economical to the form and function of the fetal pulmonary vascular state. However, to survive after birth, pulmonary arterial bifurcations must possess the functional capacity to alter their design radically in response to a broad spectrum of hemodynamic conditions (71). Whether such behavior is present in other mammalian fetal and neonatal pulmonary vascular systems, and what their properties are in the general population, requires further investigation.

We thank Jim Jones for illuminating discussions on optimality. We also thank an anonymous reviewer for suggesting that fetal bifurcation design acts to precondition fetal distal arteries to endothelial shear rates closer to their postbirth values.

This work was made possible by a grant provided by the Children’s Miracle Telethon Network.

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